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THE UTILIZATION OF GRASSES, LEGUMES AND OTHER FORAGE CROPS FOR CATTLE FEEDING IN PUERTO RICO

III. Comparison of Fertilized Guinea Grass, Para Grass and Tropical Kudzu and Guinea Grass and Tropical Kudzu.

L. RIVERA BRENES,[1] F. J. MARCHÁN[2] AND J. I. CABRERA[3]

This is the third of a series of grazing trials which started in 1945 for the evaluation of the most important pasture crops found in the Island and of those introduced.

As it is recommended by the Joint Pasture Research Committee (1) duration of pasture experiments of six to ten years should be preferred, but we think that under our conditions, where we have almost 365 grazing days, there is no need for such a long time to get reliable results. The only large variation in climate in the Island is the amount of rain. There are two or three dry months during the year, especially in the southern part of the Island. In this respect the northern part of the Island has been lucky for the past three years.

The results of these grazing trials have been reported individually in order to have the information available to the farmers as soon as possible. After the end of the fourth trial, to be finished in 1950, all the data will be compiled in one final report.

LITERATURE REVIEW

In the first grazing trial (2) the carrying capacity of Para grass-Tropical Kudzu mixture was 1 head and in the second trial (3) it was also nearly 1 head per acre. Unfertilized Guinea grass had a carrying capacity of 0.5 in the first trial and for the second trial, when it was fertilized, the carrying capacity was increased to 1.12 heads per acre.

In these two trials it was also found that fertilization increases the protein content as well as the total tonnage. The inclusion of a legume with

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the grass increased and maintained a more uniform and permanent protein content. It also increased the total tonnage of the forage.

PROCEDURE

The procedure followed has been the same as for the first trial (2). The grasses compared were Para grass-Kudzu mixture, Guinea grass-Kudzu mixture and Guinea grass alone. This time the Guinea grass was fertilized with 100 pounds of ammonium nitrate per acre at the beginning of the trial and a second application of 200 pounds of ammonium sulphate per acre three months later. To save time in the establishment of the Guinea grass-Kudzu mixture, the Guinea grass was planted in the old Kudzu stand. The land was not plowed to avoid killing the already established Kudzu, but was furrowed with a small plow at the proper distance. Pieces of roots were used and in seven months an excellent Guinea grass-Kudzu stand was obtained.

The heifers used were Holstein-Native and Brown Swiss-Native crosses, and purebred Holsteins. They were distributed among the grasses to avoid breed differences. The trial lasted 221 days, and was divided in three periods according to the number of animals grazing in each grass at one time. During the first and third periods there were two heifers per acre plot of grass and during the second period two heifers in each acre plot of Para grass-Kudzu and Guinea grass-Kudzu and one in each acre of Guinea grass alone. For the purpose of calculating gain in weight and differences in gain in weight periods one and three were put together and analyzed separately from period two. Total digestible nutrients and carrying capacity were calculated for the trial as a whole. They were calculated using the coefficients of digestibility recommended by the Pasture Research Committee (1) instead of using 72 per cent of the dry matter as in previous trials.

RESULTS AND DISCUSSION

As in previous reports weight gains and differences are presented to show probable differences in nutritive value between the grasses and legume-grass mixtures.

There was no significant difference between the average gain in weight of the animals that grazed in Para grass-Kudzu and in the Guinea grass-Kudzu mixture for the first and third periods together. The differences between the mean gain in weight in the legume grass mixtures and Guinea grass alone were significant at the 5 per cent level. No significant difference was found between the mean gains in weight during the second period. A most probable explanation to the results obtained in the second period is the following: the Guinea grass plots were fertilized with ammonium sulphate the same day (8-4-48) this second period started. The addition of

nitrogen to the soil improved the nutritive value of the grass considerably. This is sustained by the average daily gain in weight per animal. During the first and third periods together the average daily gain in Guinea grass was of 0.30 pounds and for the second period 1.19 pounds, of course, we must consider the lengths of the periods 158 and 63 days respectively which might have affected the averages. In general, the daily gain in weight per animal for the first and third periods together for all the roughages in the trial was lower than in the second period: for Para grass-Kudzu, 0.68

TABLE 1

Gain in pounds per group, mean gain per animal and least significant difference

Grasses	No. of heifers	Initial wt. of groups in lbs.	Wt. of groups at end of period in lbs.	Gain in groups in lbs.	Mean wt. in pounds per animal in lbs.	Least Significant Difference
First and third periods together 5-12-48 to 8-3-48 and 10-6-48 to 12-18-48						
PK	3	862.0	1097.0*	235.0	78.33	69.12 at 5 per cent level
PK	3	1270.0	1677.0	407.0	135.67	
GK	3	761.0	1008.0	247.0	82.33	159.60 at 1 per cent level
GK	3	1103.0	1444.0	341.0	113.67	
G	3	932.0	1052.0	120.0	40.09	
G	3	832.0	1004.0	172.0	57.33	
Second Period 8-4-48 to 10-5-48						
PK	3	1279.0	1451.0	172.0	57.33	
PK	3	1229.0	1469.0	240.0	80.00	
GK	3	1059.0	1278.0	219.0	73.00	45.75 at 5 per cent level
GK	3	1273.0	1499.0	226.0	75.33	
G	3	1004.0	1228.0	224.0	74.66	

* The final weights for these animals were taken as the initial weight plus the gain in weight during the first and third periods because the second period was analyzed separately.

pounds and 1.09 pounds per animal; and for Guinea grass-Kudzu, 0.62 pounds and 1.17 pounds respectively.

The average chemical analysis for the grass samples taken during the grazing trial are presented in table 2.

The crude protein content of the legume grass mixtures is higher than that of the grass alone, this is something to be expected, and has been true in this and the previous trials (1–3). Guinea grass received only two fertilizations instead of three, as was the case in the second experiment. The reason for this was that fertilizer was applied according to the observations made on the conditions of the grass; that is, color and growth. In trial

number 2 (3) the average analysis of protein for Guinea grass was 8.56 per cent and in this trial only 5.21 per cent, dry basis. This might be explained by the amount of fertilizer received during the experimental periods, and also to the difference in quality of the samples taken from the clippings. The grass received only two applications of nitrogen because as pointed above, observations indicated that it was green and having good growth.

The analysis of crude protein of the mixture of Guinea grass and Kudzu compares very well with the over all analysis for fertilized Guinea grass in

TABLE 2

Average analysis of the three forage crops during the whole trial in per cent dry basis

Grasses	No. of samples	% Moisture	% Ash	% Crude Protein	% Fat	% Fiber	% Nitrogen Free Extract
PK	33	75.86	8.58	9.83	1.34	32.10	48.25
GK	33	76.64	9.52	8.34	1.19	35.85	45.12
G	33	70 24	9.43	5.21	1.07	36.11	48.12

TABLE 3

*Clippings and dry matter content in pounds by periods**

Grasses	First Period		Second Period	
	lbs. Clippings	lbs. Dry matter	lbs. Clippings	lbs. Dry Matter
PK	—	—	—	—
GK	20303.0	4742.78	750.0	175.20
G	3908.0	1663.02	5929.0	1764.47

* No clippings in the third period.

trial number 2 (2); 8.34 and 8.56 per cent respectively. These results obtained emphasize the importance of the legumes in grass mixtures.

Nothing was clipped from the Para grass-Kudzu plots during the entire experimental period. Table 3 presents the amount of clippings from the other two roughages and the dry matter content. No residue was clipped during the third period in any of the roughages.

As pointed out in the procedure, the Guinea grass was planted in the old Kudzu stand. On account of that the grass was favored by a large accumulation of nitrogen in the soil. The growth obtained during the pre-experimental period was abundant. Of the 20303.0 pounds of clippings from the Guinea grass-Kudzu mixture during the first period, 18810.0

pounds were obtained after the first rotation in the three acres. From then on the heifers ate nearly all the roughage produced. This is demonstrated by the small amount obtained in the second period and by none in the third.

The effects of fertilization of the Guinea grass is demonstrated by the increase in the amount of clippings in the second period as compared with the first. An application of ammonium sulphate was made just at the start of the second period.

The calculated carrying capacity is presented in table 4.

Although the Guinea grass plots looked in good condition with only two applications of nitrogen, the carrying capacity was lower than in trial number 2 (3). Apparently two applications of nitrogen were not enough to maintain the grass as in the previous trial. Analyzing periods 1 and 3 (158 days) separately from period 2, (63 days) the carrying capacity was 0.58 and 0.92 respectively. The results in the second period demonstrate

TABLE 4

Approximate carrying capacity for the grasses in trial

Grass	Cow days	No. of days on trial	Carrying capacity
PK	169.73	221	0.77
GK	241.61	221	1.09
G	149.70	221	0.68

the favorable effects of nitrogen fertilization. The same thing has been happening with the Para grass-Kudzu mixture although the variation has not been great. The results obtained with the mixture of Guinea grass-Kudzu are encouraging; Kudzu mixes very well with the grass and there has been no sign of incompatibility, but further trials are needed to determine this.

The statistical analysis of the calculated total digestible nutrient yields is given in table 5 for the entire experimental period.

There was no significant difference at the 1 per cent level.

The mixture of Guinea grass-Kudzu was superior in T.D.N. yield to the other forages tested. This was due, of course, to the larger amount of clippings during the first period. As explained before, the Guinea grass in the mixture had the benefit of the accumulation of nitrogen in the soil which produced an exhuberant growth before the experiment started. When the heifers were put to graze on it they were not able to eat all the roughage at the time the other grass sub-plots were due for rotation. There

was no significant difference between Guinea grass alone and Para grass and Kudzu.

The rainfall figures for the eight months of the trial are shown in table 6.[4]

The water supply was more or less the same as for the previous trial. The monthly average was 7.35 inches of rainfall, which eliminates this as a factor affecting growth.

TABLE 5

Mean total digestible nutrients yield per acre and least significant difference

Grasses	T.D.N. mean yield per acre in pounds
PK	2898.33
GK	3929.66
G	2379.33
	L.S.D. 949.67 at 5% level

TABLE 6

Precipitation from May to December 1948

Month	Inches of rainfall
May	10.91
June	8.01
July	8.79
August	7.33
September	10.07
October	4.14
November	9.69
December	9.85
Total	58.79

Everything tends to indicate that the mixture of Guinea grass and Kudzu is a good combination for pasture. They mix well and apparently there is no incompatibility, but due to the fact that Guinea grass is more adapted to drier places it needs further investigation in other places around the Island.

There is no doubt as to the benefits of nitrogen fertilization of grasses; this has been demonstrated in two consecutive trials with Guinea grass But in the long-run the use of a legume should be more economical and

[4] Records kept by the Experimental Station farm.

preferable in view of the fact that the composition and yield of the forage is more uniform.

SUMMARY AND CONCLUSIONS

A third grazing trial of 221 days was conducted under the same procedure as in trial number one (2). A modification was introduced consisting in that the calculation of T.D.N. was made using the chemical analysis of the roughages, and the digestion coefficients given by the Pasture Research Committee (1).

The grasses under trial were: Para grass-Kudzu, in its third year; Guinea grass-Kudzu, in its first year; and fertilized Guinea grass, in its second year. In this experiment Guinea grass received only two applications of nitrogen instead of three as in trial number 2. This was due to the fact that according to the observations of the investigator the grass was growing well and having a nice green color. Nitrogen was applied when the leaves began turning yellowish.

Para grass-Kudzu has been uniform in performance; the carrying capacity being one head in the first trial, one head in the second and 0.77 for this trial. The carrying capacity of the mixture of Guinea grass and Kudzu was 1.09, but due to the fact that this grass was planted in the old Kudzu stand, it received the benefits of the nitrogen accumulation in the soil, producing an exhuberant growth at the beginning of the trial. As a result of this, larger amounts of residue were clipped after rotations affecting favorably the carrying capacity. Although the results are encouraging, further investigations are needed to make definite recommendations. Guinea grass alone had a carrying capacity of 0.68. It was lower than in the previous trial. The difference can be attributed to the difference in nitrogen supplied, as nitrogen increases the amount of roughage produced and the nutritive value of the forage.

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CAROTENOIDS AND VITAMIN A IN THE BLOOD OF DAIRY CALVES IN PUERTO RICO

F. J. MARCHÁN,[1] L. RIVERA BRENES[2] AND L. F. COLÓN[3]

During the past years the Agricultural Experiment Station of the University of Puerto Rico has undertaken a series of fundamental studies in the nutrition of the dairy cow in this Island with the purpose of determining which nutritive factors are operating in such a way as to limit milk production. The low production of the dairy cow in Puerto Rico might possibly be the result of the poor growth and development of our dairy calves, which result in undersized cows with a lower productive capacity. Observations made at this Station[4] show that the growth of the Holstein calves does not compare favorably with the Missouri standards (9) for that breed. Calf losses before three months of age have been heavy in recent years; 28 per cent in 1946, 19 per cent in 1947 and 29 per cent in 1948. These deaths have been due to scours, pneumonia and unknown causes, perhaps nutritional deficiencies. Although no symptoms of vitamin-A deficiency had been observed this study was started in order to ascertain whether or not our calves were being supplied with adequate amounts of this nutrient whose importance in calf nutrition has been definitely established.

Modern feeding and managerial practices among dairy calves have been one of the major obstacles to herd improvement. Moore and Berry (7) found that when calves were reared according to present-day methods of limited whole-milk feeding, the vitamin A content of the blood plasma from birth to four months of age was in the deficient range, as judged by blood values of vitamin-A deficient calves 4 to 14 months of age. Wise et al. (13) found that the vitamin-A content in the blood serum of calves at five weeks of age was 50 per cent lower than it was at the colostrum feeding period. Moore et al. (8) reported that the vitamin-A content of the blood plasma of dairy calves reared on a limited whole milk program was one third lower than that of beef calves of the same age that were permitted to suckle and thus obtained considerable more milk. Jacobson et al. (3) reported that calves permitted to run with their dams on pasture required four months on a vitamin-A deficient ration to deplete their reserves, while those reared according to present methods of limited whole milk feeding with hay of above average quality, required a depletion time of 2 to 4 weeks. Wise et al. (13) at the Kansas Agricultural Experiment Station have shown that

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[4] Unpublished data, Agricultural Experiment Station, Rio Piedras, Puerto Rico.

during the interim between colostrum ingestion and effective hay consumption there was a marked decrease of vitamin-A and carotene in the blood of calves and they recommended vitamin-A supplementation during this period. With this information at hand, it was decided to study the vitamin-A and carotene content of the blood of a group of dairy calves, compare them with those in the States and determine whether or not it was necessary to supplement the calf ration.

METHODS

Twelve calves born at this Station from October 9, 1948 to March 21, 1949 were used in this study. The following breeds and crosses were represented: purebred Holstein, purebred Natives, grade Holsteins, Brown Swiss x Native and Holstein x Native. Immediately after birth and before ingestion of colostrum, the calves were bled and the carotene and vitamin-A determined by the method of Kimble (5). Thereafter determinations were made 24 and 72 hours after birth and when the calves were one, two, three and four months old. After the first sample of blood was drawn the calves were allowed to suckle from their dams, with whom they were kept for the first three days. Then they were removed to individual pens and fed whole milk from the Station herd at the rate of 10 per cent of their body weight. Five or six days after birth the calves were offered some grain and green Merker grass (*Pennisetum purpureum* var. Merkeri) with an average carotene content of 11.0 mg./lb. on a fresh basis.[5] When the calves were one month old they were given reconstituted skimmilk until six-months of age with the exception of calves number four and number eleven which were given powdered skimmilk at the same rate and of calf number five which was changed to a yeast ration when two months old.

RESULTS AND DISCUSSION

Table 1 presents the carotene and vitamin-A values obtained for the calves under study.

A summary of the statistical analysis of the data is presented in tables 2 and 3.

Statistical analysis of the data showed that there is a significant difference in carotene and vitamin-A content between ages, the deviations from linearity being not significant (figure 1). Results obtained by other workers, Hansen, Phillips and Rupel (1), Hansen, Phillips and Smith (2) and Moore and Berry (6) show that the concentration of carotenoids and vitamin-A in the blood serum of dairy calves were low at birth, increased markedly after colostrum ingestion and then declined until the fifth week, when they started to increase again. These results were confirmed by Wise et

[5] Unpublished data, Agricultural Experiment Station, Río Piedras, Puerto Rico.

TABLE 1

Calf No.	Breed	Birth		24 hrs.		72 hrs.		1 month		2 months		3 months		4 months	
		Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A
1	H x N	4.8	6.0	6.0	6.0	10.5	13.5	24.0	39.0	—	—	—	—	—	—
2	H x N	3.6	12.0	10.5	21.0	15.0	30.0	15.0	4.05	90.0	66.0	102.0	22.5	183.0	34.5
3	BS x N	16.5	16.5	18.0	24.0	27.0	36.0	—	—	—	—	—	—	—	—
4	BS x N	7.5	7.5	18.0	21.0	15.0	7.5	18.0	7.5	37.5	39.0	126.0	29.3	126.0	43.5
5	GH	4.5	6.0	15.0	21.0	33.0	16.5	27.0	18.9	50.0	34.5	93.0	21.0	112.5	27.0
6	GH	3.0	6.5	—	—	—	—	45.0	16.5	144.0	33.0	153.0	33.8	—	—
7	H	12.0	19.5	12.0	36.0	15.0	22.5	30.0	34.5	42.0	19.6	114.0	27.0	129.0	27.0
8	H	12.0	12.0	15.0	15.0	21.4	8.3	48.0	21.0	42.0	27.0	168.0	39.8	195.0	40.0
9	BS x N	6.0	19.5	15.0	19.5	15.0	12.0	19.5	10.5	57.0	19.5	84.0	26.3	105.0	30.0
10	H	1.5	7.5	9.0	16.5	20.0	28.3	15.0	16.5	24.0	24.0	—	—	—	—
11	N	6.0	12.0	12.0	19.5	30.0	21.8	42.0	7.5	45.0	25.2	84.0	31.5	100.0	32.0
12	GH	3.0	4.5	13.5	12.0	12.0	21.8	51.0	15.0	—	—	—	—	—	—
Average		6.7	10.8	13.1	19.2	19.4	19.8	30.4	20.7	59.1	32.0	116.0	28.9	135.8	33.4
Standard Deviation		4.54	5.34	3.68	7.52	7.55	9.21	13.7	12.0	36.6	14.4	31.5	6.14	38.0	6.4

Carotene and vitamin-A expressed as micrograms/100 ml. of plasma.
Calves number 1 and number 3 died at 5 weeks of age.
H—Holstein.
N—Native.
BS—Brown Swiss.
GH—Grade Holstein.

al. (13) who recommended vitamin-A supplementation during this period. The information obtained from our study shows that although the increase in carotene and vitamin-A after colostrum ingestion is not so marked as compared to the States, there is no need of supplementation since these constituents continue increasing steadily up to four months of age, the period of decline reported in the literature being totally absent. This continuous increase in carotene and vitamin-A can probably be explained on the basis of a larger reserve stored up in the liver of the calf during

TABLE 2

Source of variation	DF	SS	Mean Square	F value
Within ages (error)	62	29,142.12	470.03	
Linear regression	1	132,973.56		
Deviation from linearity	5	(4,509.85)	901.97	1.91
Age	6	137,483.41	22,913.90	48.74
Total	68	166,625.53		

TABLE 3

Summary of statistical analysis for vitamin-A

Source of variation	DF	SS	Mean Square	F value
Within ages (error)	62	5,333.57	86.03	
Linear regression	1	2,912.80		
Deviation from linearity	5	(934.55)	186.91	2.17
Age	6	3,847.35	641.22	7.45
Total	68	9,180.92		

prenatal development due to the fact that their dams have been on green pasture all the year round.

In its report of 1945 the National Research Council (9) recommended for dairy cows a daily allowance of 90 mg. of carotene during the last 6 to 12 weeks before parturition. The fact that carotene requirements are lower at higher temperatures (4) suggests that in the tropics the requirements might be less. As our cows eat daily about 7 pounds of Merker grass per 100 pounds of live weight with an average content of 11.0 mg. of carotene per pound, their intake is considerably above their requirements. This permits the prenatal calf to store an ample supply in its liver, which can be used in the critical period comprised after colostrum ingestion and before effective grass consumption.

The carotene and vitamin-A values obtained at birth 1, 2, 3 and 4 months were compared with those given by Warner (12), Spielman (11), Wise (13) and Keener (4) for normal well-developed calves and in all cases our values compared favorably with those given by these authors. The results obtained are a strong indication that up to 4 months of age, vitamin-A is not a factor affecting the growth and development of our calves. It is logical to believe that high mortality and the slow rate of growth are probably due to some other causes.

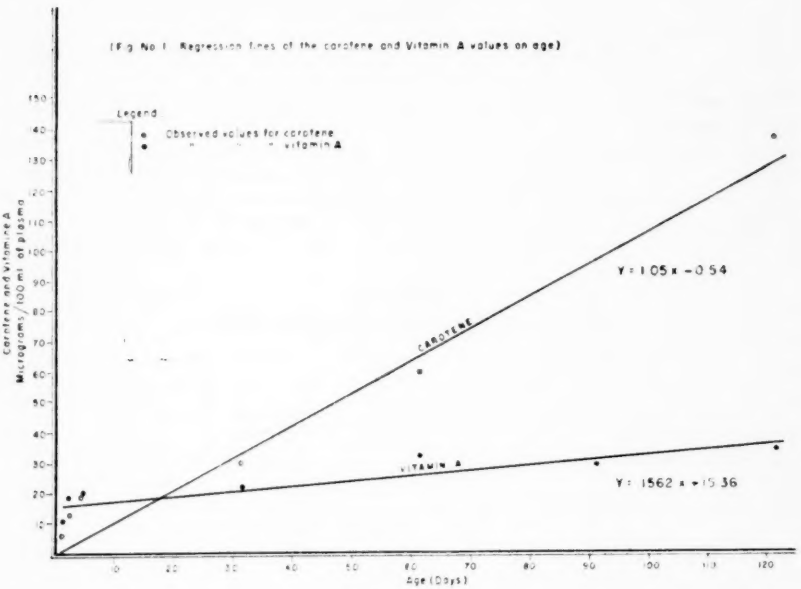
(Fig. No 1 Regression lines of the carotene and Vitamin A values on age)
Legend
Observed values for carotene
vitamin A
Carotene and Vitamin-A
Micrograms/100 ml of plasma
Y = 1.05 x - 0.54
CAROTENE
VITAMIN A
Y = 1562 x + 15.36
Age (Days)

FIG. 1. Regression lines of the carotene and Vitamin A values on age.

SUMMARY AND CONCLUSIONS

The carotene and vitamin-A content of the blood of a group of dairy calves was determined at birth, before ingestion of colostrum, 24 and 72 hours after birth, and thereafter at monthly intervals until 4 months of age.

There was a significant difference in carotene and vitamin-A between ages, the relationship being best described by a straight line. Carotene and vitamin A values compared favorably with those given in the United States for normal calves.

Supplementation with vitamin-A during the period comprised between

the third day after birth and effective grass consumption (5 weeks of age) is considered unnecessary.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Mr. Antonio González Chapel for his help in the statistical analysis.

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RIBOFLAVIN IN EGGS FROM HENS OF DIFFERENT BREEDS RAISED IN PUERTO RICO*

CONRADO F. ASENJO [1]

Previous work from this laboratory (1) has indicated a wide variation in the riboflavin content of eggs of unknown history, purchased at different market-places in San Juan, Puerto Rico. Suspecting that the breed of the hens might account at least, in part, for such variability, it was decided to determine this factor through the riboflavin assay of eggs obtained from known breeds raised and fed under the same conditions. The result of this investigation, as well as a discussion of the literature related to riboflavin in eggs, is reported in this paper.

In 1929, Roscoe (2) (3) and co-workers showed that egg yolk and egg white contained vitamin G. Roscoe (4) in 1931, found fresh egg yolk richer in vitamin G than fresh egg white; however, on dried basis he observed that egg white was the richer of the two. In 1933, Kuhn, Gyorgy, and Wagner-Jauregg (5) demonstrated that riboflavin from egg (ovoflavin) had vitamin G activity; later, it was found that riboflavin and vitamin G were the same substance.

In 1934, Von Euler, Adler, and Schlotzer (6) determined, colorimetrically, the riboflavin content of egg yolk, egg white, and whole egg reporting the following values per g. of material: yolk—5.5 ug., white—4.5, and whole egg—4.8 ug. of riboflavin, respectively. In 1939, Bauernfeind and Norris (7) assayed eggs by the fluorometric method. These eggs came from hens receiving a ration of 1 ug. of riboflavin per g. of ration and were found to contain 1.1 ug. of riboflavin per g. of fresh yolk and 0.65 ug. of riboflavin per g. of fresh egg white. Winter and Bethke assayed dried eggs biologically and reported values equivalent to 6.4 to 20.0 ug. of riboflavin per g. of whole dried egg. Using the microbiological procedure, Snell and Strong (8) in 1939, found that fresh egg yolk contained 7.6 ug. and egg white, 3.1 ug., of riboflavin per g. Lepkovsky, Taylor, Jukes, and Almquist, (9) in 1938; Hunt, Winter, and Bethke, (10) in 1939; and Engel, Phillips, and Halpin, (11) in 1940, observed that within certain limits the level of riboflavin in the ration increased the riboflavin in content of the eggs.

In 1940, Norris and Bauerfeind (12) analyzed, fluorometrically, farm eggs from hens receiving various types of egg mash and found 2.39 ug. of

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[1] From the Department of Chemistry, School of Tropical Medicine, with the technical assistance of Mercedes M. de Gracia and Hilda García de la Noceda.

riboflavin per g. in the yolk, 1.90 ug. in the white, and 2.07 in the whole egg. These same investigators reported the following values in farm eggs from hens receiving breeding mash that generally contained more riboflavin than egg mash: egg—2.85 ug.; egg white—2.29 ug., and whole egg—2.48 ug. of riboflavin per g. In 1941, Snell and Quarles (13) recorded a value of 2.0 ug. of riboflavin per g. for fresh whole egg when assayed by the microbiological procedure. Peterson, Dearstyne, Comstock, and Weldon, (14) in 1945, separated the white and yolk of hard boiled eggs and determined the riboflavin content fluorometrically, as follows: egg yolk per g., 4.07 ug.; egg white per g., 2.67 ug.; in the whole egg the riboflavin content was 3.18 ug. per g.

The following year Jackson, Drake, Slinger, Evans, and Pacock (15) reported the riboflavin content of eggs from twelve different breeds fed a mash containing 3.1 u. of riboflavin. Riboflavin values ranged from 3.71 ug. to 2.91 ug., with an average of 3.36 ug. of riboflavin per g. Among the eggs assayed there were some from New Hampshire and White Leghorn breeds reported to contain 3.15 ug. and 3.65 ug. of riboflavin per g., respectively.

The most recent compilation of the U. S. Department of Agriculture and the National Research Council (1945) (16) gives a riboflavin value of 3.4 ug. per g. of fresh whole egg and about 173 ug. per average egg.

MATERIAL AND METHODS

The eggs utilized in this study came from the La Plata Animal Production Sub-station of the Agricultural Experiment Station of the University of Puerto Rico, near Cayey, a town located in the center of the Island at an altitude of 1,233 feet above sea level. Eggs from three different breeds were studied: New Hampshire, White Leghorn, and native. The pollorum reaction of the three breeds were 27.70, 21.07 and 6.84 per cent, respectively. These fowl were kept under identical conditions in the standard chicken house, with mesh wire floors so that there was little access to the feces. The hens received a laying mash having an average of 2.63 (range, 2.60—2.65 ug.) ug. of riboflavin per g. Ration samples were analyzed by the same procedure used for the eggs, which had been collected during an interval of a year by alternating the three breeds.

Analysis was performed within the same week that the eggs were laid, at which time the weight of the whole edible part, as well as the individual weight of both white and yolk, was recorded. Samples were prepared in a Waring blender adding a known amount of water and portions of this mixture used for riboflavin determinations by the microbiological procedure of Snell and Strong (8). Each sample was tested at four different levels (using duplicate tubes at each level). Those results falling outside the

range 0.05 to 0.25 ug. of riboflavin per tube were discarded. If the maximal variation of the remaining values was not over 10 per cent, all values were averaged to give the final result for that particular sample. The results obtained are presented in table 1.

TABLE 1

Result of analysis

Item	Breed		
	New Hampshire	White Leghorn	Native
1. Weight of edible part of egg, g. (ave. 12 eggs)	49.4 (2.0)*	53.2 (1.9)	40.3 (1.8)
2. Per cent yolk (ave. 12 eggs)	36.1 (2.2)	38.3 (1.6)	42.9 (2.2)
3. Per cent white (ave. 12 eggs)	63.9 (2.2)	61.7 (1.6)	57.1 (2.2)
4. ug. riboflavin in yolk (ave. 36, 24 and 24 eggs, respectively)	6.96 (1.13)	6.30 (1.10)	4.96 (0.91)
5. ug. riboflavin in white (ave. 36, 24 and 24 eggs, respectively)	4.20 (0.89)	3.70 (0.85)	2.68 (0.74)
6. ug. riboflavin per g. of whole egg edible portion calculated from 2, 3, 4, and 5	5.19	4.69	3.65
7. ug. riboflavin per g. of whole egg edible portion determined directly (ave. 12 eggs)	5.17 (0.72)	4.70 (0.63)	4.13 (0.63)
8. Average of 6 and 7	5.18	4.70	3.89
9. ug. riboflavin per egg using the average of 6 and 7	256	250	157

* Numbers in parenthesis are standard deviation.

DISCUSSION

The results appearing in table 1 indicate that eggs from native hens contain 3.89 ug. per g. of edible portion. This is less riboflavin than in eggs from New Hampshire and White Leghorn hens kept under the same conditions and fed the same ration. The difference between native eggs and those of hens from other breeds is more pronounced when the total riboflavin

content per egg is compared. This is due to the fact that native eggs contain a considerably less amount of edible matter.

The per gram content of riboflavin for fresh yolk, white, and whole eggs, found in the three breeds studied, was considerably higher than the average reported by other investigators for eggs from continental breeds raised in northern latitudes. Jackson *et al.* (15) found that the riboflavin content of eggs from New Hampshire and White Leghorn hens, raised in Canada and fed mash containing 3.1 ug. of riboflavin per g., was 3.15 and 3.65 ug. per g. of whole fresh egg, respectively, as determined by a modification of the fluorometric procedure of Hudson and Norris (17). These values were more than a microgram below those found for eggs from hens of the same breeds but raised in Puerto Rico. While the values are not strictly comparable, since they were obtained by two different methods of analysis, it may be noted that even the native eggs exhibited a higher content of riboflavin per g. of fresh whole egg than any of those reported by the Canadian investigators.

It is noteworthy that Snell and Strong (15), using the same procedure employed in this laboratory, found that eggs from continental breeds averaged values of 7.1 ug. and 3.1 ug. per g. for fresh yolk and white, respectively. These values are more or less like the ones found by the writers.

SUMMARY

The riboflavin content of eggs from New Hampshire, White Leghorn and native hens, raised in Puerto Rico, was found to be 5.18 ug., 4.70 ug., and 3.89 ug. per g. of whole fresh egg, respectively. The hens all received a mash containing an average of 2.63 ug. of riboflavin per g.

ACKNOWLEDGMENT

The author is indebted to Dr. F. E. Armstrong of the Agricultural Experiment Station for his cooperation in this project.

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ON THE OCCURRENCE OF A TOMATO DISEASE IN PUERTO RICO RESEMBLING BIG BUD (CHLOROGENOUS AUSTRALIENSIS Hol.)

L. A. ALVAREZ-GARCIA[1] AND JOSE ADSUAR[2]

INTRODUCTION

This paper is a preliminary report on the occurrence of a tomato disease in Puerto Rico resembling Big Bud as described in Australia (1).

Big Bud of tomato in Puerto Rico was reported by the senior author since January 1945. The disease appeared among tomato plants grown experimentally in the fields of the Station.

The amount of diseased plants at that time was very small, 16 plants out of 3040, that is, approximately 0.5%, showed disease symptoms. During the fall and winter months of October 1945 to January 1946, 31 plants out of 1,800 or 1.6% appeared diseased and in the January–April, 1946 growing season, 236 plants out of 1,600 or 14% showed various stages of Big Bud infection.

Rainfall registered during each corresponding growing season was:

January–April, 1945	6.5 inches
October 1945–January, 1946	5.5 inches
January–April, 1946	3.5 inches

There was apparently a negative correlation between amount of rainfall and increment of the disease. In all probability this could be ascribed to a gradual increase in virus reservoirs or in a greater population of a possible insect vector associated with the transmission of the viral agent (fig. 1).

SYMPTOMS OF THE DISEASE

The first noticeable symptom in tomato is characterized by a virescence of the young leaves and a peculiar stiffness and retardation of development of the young shoots. A rolling of the margins of older leaves is also very conspicuous (fig. 2).

When infection occurs in young tomato plants, the characteristic, generalized symptom is a marked retardation of growth of the plant, thickening of stem and branches, and proliferation of leaf buds, with subsequent atrophy of the newly formed leaves (fig. 3). The plant if infected very young ceases to grow, no leaf development occurs and the thickened stem

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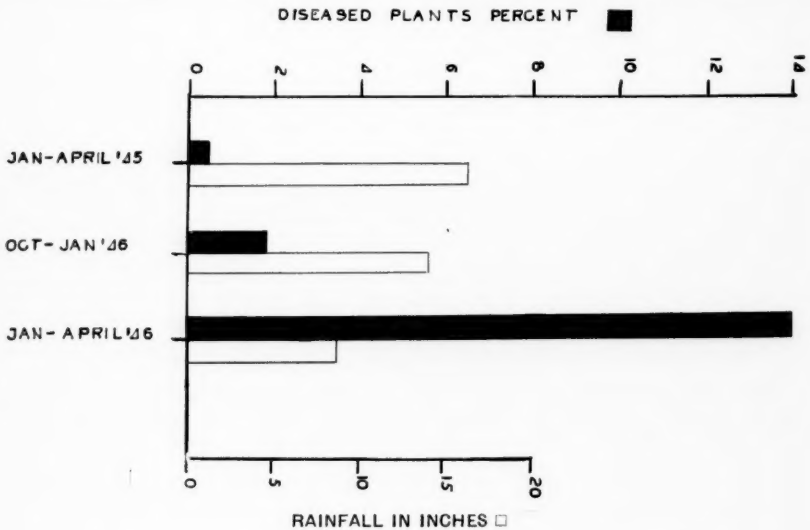

FIG. 1. Per cent of Big Bud diseased plants and number of inches of rainfall observed during the various growing seasons.

FIG. 2. Diseased tomato plants of the variety Bounty, showing leaf virescence, atrophy and stiffness of young leaves, and rolling of old leaves. Plant at the left showing infection. Plant at the right showing early infection at time of flowering. Note upright and vertical flower truss, enlargement of sepals and abortive fruits.

and branches become yellowish. In extreme cases the whole plant structure is completely denuded of foliage (fig. 4).

When infection occurs at flowering time, the young flowers show very marked enlargement of the sepals which become thickened, somewhat oval in cross section and flattened on the upper side. The flowers assume an abnormal, upright position in contrast to the curved downward position

FIG. 3. Enlarged view of apical growth of an early infected tomato plant showing somewhat enlarged flower buds, proliferation of leaf buds, atrophy and stiffness of young leaves.

of flowers in normal plants (fig. 5). Young infected flowers show atrophy of stamens and ovary and are sterile; if fruit has already been formed before infection, its growth is checked and necrosis follows. Enlargement of the calyx, as reported from Australia, does not occur.

TRANSMISSION STUDIES

Attempts by various workers to transmit mechanically the tomato Big Bud virus (*C. australiensis*) have met with failure. Transmission has been possible through grafting of buds and scions taken from diseased tomato

plants and also by means of the leaf-hopper, *Thamnotettix argentata* Evans (2).

Cranberry false blossom virus (*C. vaccinnii* Hol.) when transferred to tomato plants, produces a symptomatology resembling very closely that

FIG. 4. Two young tomato plants, variety Marglobe, infected with Big Bud virus. Plants at the left showing normal growth. Plant at the right, early infected and showing marked dwarfiness. Both plants were of the same age.

of tomato Big Bud. This viral entity has been transferred either by grafting, by way of the parasitic phanerogam (*Cuscuta campestris* Yuncker L.), or by the insect vector, *Euscelis striatulus* (Fall). This type of Big Bud virus has also been transmitted by grafting to *Vinca rosea* L., tomato, potato, Turkish tobacco and *Nicotiana glutinosa* L. (3).

Experiments initiated at this Station demonstrated the unsuccessful mechanical transmission of the virus. Transfer of the infectious entity has been obtained only by means of grafting. Experiments conducted during March and April 1945, by budding, side grafting and inarching, revealed that transmission is always obtained when union of scion and host tissue has occurred. The amount of infection fluctuated from 60 to 80% in young, fast growing tomato plants, variety Marglobe. Best results were obtained when buds were taken from diseased plants showing well advanced symptoms of the Big Bud disease. Transmission through *C. filiformis*, a parasite

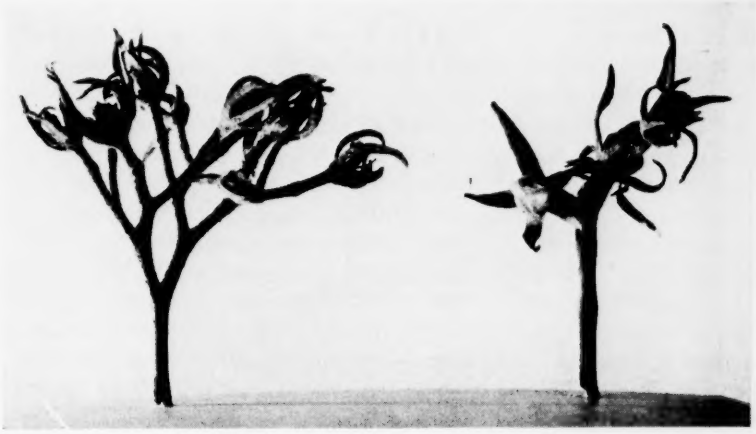

FIG. 5. Flower truss showing abortive fruits

rather endemic in the Island and by way of possible insect vectors has, so far, met with failure.

Symptoms of infection in grafted plants were noticeable within 30 to 40 days after grafting. This conforms very closely with the incubation period (28 to 56 days) for *C. australiensis* reported by Samuel, Bald and Earley in Australia.

CONCLUSIONS AND SUMMARY

The Puerto Rican type of tomato Big Bud disease is similar to that reported from Australia.

The main difference in symptomatology lies in the absence of a pronounced bladder-like hypertrophy of the calyx of virus invaded flowers. Other slight differences between the Puerto Rican and Australian type of

Big Bud could be possibly due to:

1) the existence of two distinct strains of the virus.
2) the influence of local environment on symptom manifestation.

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CONTROL OF THE SOIL-INHABITING GRUBS OF PUERTO RICO[1]

GEORGE N. WOLCOTT[2]

The roots of agricultural crops in Puerto Rico are attacked by two kinds of soil-inhabiting grubs, either one of which may cause an immense amount of damage if present in appreciable numbers. The common white grubs of the coastal regions are the immature stage of the May beetles or "caculos", described by Mr. E. G. Smyth as *Phyllophaga portoricensis* and *P. vandinei*. They have six legs, and their bodies are bent double, so that the hind end is opposite the powerful jaws with which they eat the roots of plants.

The grub of the "vaquita" has an oval body, and as it possesses no legs, progress thru the soil depends on its rapid powerful muscular contractions. The injury which these legless grubs cause to a plant is mainly from their habit of burrowing to the inside of its subterranean portion, and feeding on its interior. Apparently they are quite as omnivorous in their feeding habits as are white grubs, having been noted attacking the roots of weeds and forest trees, such as mahogany seedlings in nurseries, as well as those of economic plants. White grubs attacking sugar-cane first eat the rootlets and then chew into the root-stalk, but vaquita grubs when growing most rapidly burrow inside the root-stalk and feed within. They have thus received the name of sugar-cane weevil root-stalk borer, the adults being the striped weevils scientifically known as *Diaprepes abbreviata* (Linnaeus).

Despite very heavy larval mortality, survival in sufficient numbers to be a very serious economic pest is assured by the five thousand (more or less) eggs which are laid by the female weevil (Wolcott 1936). The legless little grubs, which hatch seven days after oviposition, appear to have a very tenuous hold on existence, for despite their vigor and constant activity, few of them even attempt to eat, and of the survivors, growth before transformation to second instar is slow as compared with that in the instars immediately following. In the first, second and third instars, the grubs feed on very small roots or on root hairs, showing little or none of the tendency to burrow into the larger roots which appears in the fourth instar. That is, up to the beginning of the fourth instar, they are free-moving in the soil, completely unprotected against any toxic substance which may be present. Thus if any grubs survive in soil to which toxicants have been added, their survival proves that the chemical is not present in sufficient amount to cause mortality.

After the beginning of the fourth instar and up to the time that the

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grubs have attained full size, they are increasingly protected against the effect of any chemical in the soil by their habit of burrowing inside the grain of corn, or root-stalk of sugar-cane, or comparatively large root or corm or tuber of any plant within which and upon which they may be feeding. In the diapause period before pupation, when they vigorously burrow about thru the soil without any apparent purposes, they are again fully exposed to the action of any insecticidal chemical that may be present in the soil, but naturally during this period of their life-history are much more resistant to extraneous chemicals than as just-hatched or very small grubs.

Laboratory experiments conducted with large or fully-grown *Diaprepes* grubs in the same soil and paralleling similar tests with third (last) instar white grubs of *Phyllophaga portoricensis* Smyth and *P. vandinei* Smyth, in their susceptibility to several of the newer insecticides, gave most inconclusive and indeed at times quite contradictory results. The tests with the third instar white grubs made possible very definite recommendations as to the effective amount per acre to be applied of aldrin (Hyman 118), the gamma isomer of benzene hexachloride (lindane), rhothane or DDD, and DDT (Wolcott 1950). Even after being mixed with the soil for a year both aldrin and lindane showed little or no decrease in toxicity to white grubs, indicating that field applications at the rate of as little as two pounds per acre should result in commercial control of this pest.

By comparison, the tests with *Diaprepes* grubs were so unsatisfactory as to suggest that the resistance to chemicals of these grubs in the diapause or pre-diapause stage is ever so much greater than of other grubs of approximately the same size which are still eating and growing. To attain some degree of uniformity, all grubs being tested should be in the same instar, presumably insuring that they have approximately the same susceptibility to toxic substances. The most obvious way of starting with all grubs in the same instar is to use those which have just hatched. If, despite high larval mortality due to natural causes, some of these can be reared to fourth instar (at which time they cease to be subject to the effect of chemicals mixed with the soil because of their habit on burrowing within the host), this furnishes quite definite proof of the lack of toxicity of the chemical at that rate of concentration in the soil. Admittedly this begs the question of control of large grubs, but as they have already caused most of the injury possible, their destruction is really not as important as that of killing the small grubs before they have begun to burrow into the roots of sugar-cane or other crop.

Oviposition by females of *Diaprepes abbreviata* occurs during every month in the year, reaching a maximum during May and June, but because of high parasitism by *Tetrastichus haitiensis* Gahan during these months,

it is doubtful if many more grubs reach the soil at this time than when egg-clusters are less numerous. The result is a more or less constant reinfestation of the soil, with no well-defined season when maximum injury from the grubs may be anticipated, to prevent which a toxicant might be applied to the soil. Moreover, few cane growers would thus anticipate damage by *Diaprepes* grubs, but might be expected to apply insecticides after injury had begun to be noticeable, and the specific grubs causing it had already reached their diapause stage. Thus the residual effect of the chemical on the just-hatched grubs for some months or years after application to the soil will indicate a more practical method of keeping the soil free of grubs for an extended period, especially if the insecticide is also toxic to white grubs for an equally long time. This extended explanation is given that it may be apparent why the tests about to be described were conducted, and why the methods adopted were used.

One ounce tin salve boxes, and later two ounce tin salve boxes, were filled with earth from the much larger cans in which the tests had been conducted with white grubs, in each of which cans the amount of insecticide mixed with the soil was computed on the basis of field applications of pounds per acre. As this soil had been most thoroly mixed with the insecticide originally, and had been repeatedly mixed subsequently every time the large cans were dumped for determination of white grub mortality and and for replanting with corn, cucumbers and/or papayas, the small samples of earth extracted for test with the just-hatched *Diaprepes* grubs in the salve boxes may be considered typical. In every case, the results obtained with *Diaprepes* grubs were consistent with each other, indicating that even these small samples contained the proper proportion of insecticide. One grain of corn and four cucumber seeds were planted in the earth in each one ounce salve box, and in the two ounce tins, four grains of corn. Water was added with a medicine dropper so that the soil would be of optimum moisture content both for the germination of the seeds and for the survival of the grubs. Each day for a week, approximately equal numbers of the *Diaprepes* grubs which had hatched in the preceding 24 hours were placed on the surface of the soil in each salve box. In the later tests, all grubs that hatched in the preceding 24 hours were placed in the cans with earth mixed with different dilutions of only one chemical, that the date of starting the test might be the same for all. However, to make sure that conditions would be equal for all, subsequent additions might be made when the earth had dried somewhat as the corn germinated. At weekly intervals, or oftener if the corn seedlings tended to push up the cover of the salve box, the entire contents was dumped, the presence or absence of grubs noted, their growth and stage of development as indicated by head-size, and grubs and soil replaced, with fresh seeds and an appropriate amount of water

added. The tests terminated when at least one grub had reached fourth instar, as indicated by the size of its head capsule, as well as by its change of habit of burrowing into the grain of corn rather than continuing to feed on the roots and root-hairs.

RESULTS

In the winter rearing experiments (November 1949 to January 1950), by the 27th day after hatching no live grubs could be found in the earth containing 0.5 aldrin, and by the 41st day, no live grubs could be found in the earth with 1 lb. per acre of aldrin (Hyman 118). However, one grub in 4th instar, transferred from 1 lb. gamma isomer to 1 lb. Hyman 118, did survive for over two weeks, feeding in the corn grain, but having transformed to 5th instar, was dead by the end of the third week. The only other test resulting in the mortality of the grubs was that containing 10 lbs. per acre of rhothane (DDD), of which some grubs reached 2nd instar 33 days after hatching, but all were dead and covered with spores of the green Muscardine (*Metarrhizium anisopliae*) by the 41st day.

In the winter rearing tests, grubs had transformed to 4th instar by the 33d day in the cane containing earth mixed with 1 lb. of gamma isomer per acre, and with 1 lb. and 2 lbs. of chlordan. By the 41st day, grubs had transformed to 4th instar in 2 lbs. of gamma isomer per acre, in 10 lbs. of DDT per acre, and in the check. After 47 days, grubs had transformed to 4th instar in earth containing 10 lbs. per acre of methoxychlor, 10 lbs. of zinc dimethyldithiocarbamate, 10 lbs. CPR (U. S. Industrial Chemicals), and 10 lbs. of ryania.

The poor showing of some of these chemicals was not unanticipated, as they had been similarly ineffective against white grubs. But the gamma isomer of benzene hexachloride had been almost as effective as aldrin in killing white grubs, and in May 1950 another series of tests was started to determine the concentration at which gamma isomer would be toxic. As in the winter tests, but more rapidly because of somewhat higher temperatures, all the grubs died in the cans containing earth mixed with aldrin (Hyman 118) at 5 lbs., 2 lbs., 1 lb. and 0.5 lb. per acre, none being noted after the second week. By the 16th day, no live grubs could be found in the earth containing 10 lbs. of chlordan per acre, and by the 20th day none in the earth with 5 lbs. of chlordan. However, some grubs in the 1 lb. per acre chlordan had successfully transformed to 4th instar by the 20th day after hatching; by the 32nd day, some had reached 4th instar in the earth containing 2 lbs. chlordan per acre.

During the summer rearing tests, some grubs were still alive in 2nd instar by the 19th day after hatching in the earth containing 25 lbs. and 20 lbs. of DDT per acre, but by the 29th day all were dead.

By the 24th day after hatching, grubs in the earth containing 1 lb. per acre of gamma isomer of benzene hexachloride had transformed to 4th instar; by the 31st day, six grubs had reached 4th instar in the earth containing 5 lbs. of gamma isomer per acre, and by the 38th day, one grub had reached 4th instar in the earth with 10 lbs. of gamma isomer per acre.

CONCLUSION

Gamma isomer of benzene hexachloride is almost as effective in killing third instar white grubs (*Phyllophaga* spp.) in Puerto Rico as is aldrin (Hyman 118), applications of either at the rate of 2 lbs. per acre resulting in commercial control. It is much inferior in causing the mortality of just-hatched weevil grubs of *Diaprepes abbreviata* L., in laboratory tests, merely delaying the rapidity of development at 10 lbs. per acre. Aldrin in all tests killed all *Diaprepes* grubs at one-twentieth of this concentration, at 0.5 lbs. per acre.

As neither the amount of gamma isomer necessary to kill *Diaprepes* grubs, nor the maximum amount of aldrin in soil in which they can survive was determined, the disparity in effectiveness of the two insecticides is even greater than these tests indicate.

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THE TERMITE RESISTANCE OF PINOSYLVIN, STILBENE AND OTHER NEW INSECTICIDES[1]

GEORGE N. WOLCOTT[2]

The natural resistance to termite attack of some woods has long been known, but chemical analyses, to determine the specific chemical constituents responsible, have rarely been made. Until very recently almost the only exception seems to have been in the case of East Indian teak, *Tectona grandis* L. The well-known resistance to weathering, decay and insect attack of teakwood is apparently due to the presence of beta-methylanthraquinone (tectoquinone). A ten minute submersion in a 1% solution in acetone of tectoquinone will indefinitely protect susceptible woods against attack by the West Indian dry-wood termite, *Cryptotermes brevis* Walker, (Wolcott 1946). The partly-eaten keys of a piano (of which one key had already been replaced with a duplicate made of mahogany), heavily infested with these termites, have thus been successfully protected against further injury, despite the fact that the untreated remainder of the piano is falling to pieces, so rapidly is its oak case being devoured.

In the East Indies, tectoquinone is extracted from teakwood waste, and in the United States is synthesized by Eastman, but in neither case is cost of production sufficiently low that this chemical can be recommended for extensive commercial adoption. Tests of many other quinones and related compounds have indicated none that is even as effective in protection against termite attack as is this natural constituent of teakwood (Wolcott 1947).

Pinosylvin (3,5-hydroxystilbene) has been extracted from the heartwood of Scotch pine by Dr. Holger Erdtman, in charge of the Division of Organic Chemistry, the Royal Institute of Technology, Stockholm, Sweden. It has a diphenolic structure which "suggests that pinosylvin might be the main cause of the well-known great durability of pine heartwood" (Erdtman 1949). The sample very graciously submitted for test consisted of light, silvery flakes, of a delightful piney odor for some people, odorless to others, which floated on the surface of water, but dissolved instantly in alcohol or acetone like the first snowflakes of winter in a forest pool, and this solution could be diluted indefinitely with water without precipitation of the crystals. Using a standardized technique, fresh samples of flamboyán, *Delonix regia* (Bojer) Raf., were submerged for ten minutes in solutions of pinosylvin at 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02% and 0.01% on July 6th, 1950. The next day, the sample impregnated with 0.01% pinosylvin was placed

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with fresh termites in a petri dish with similar samples of flamboyán treated with other chemicals. On the following morning half of the termites were dead or immobilized. For nearly two months, no termites have been able to live longer than two or three days in the petri dish containing this sample, but eventually it lost its toxicity. Five months after impregnation, a slight gouge was eaten in it, but in succeeding months, no fresh termites have enlarged this inconclusive initial attack.

Some inorganic chemicals, notably red mercuric iodide (Wolcott 1943), are initially very toxic to termites, but wood impregnated with them rapidly ceases to be either toxic or repellent. Many of the organic chemicals are also initially toxic, most notably zinc and copper dimethyldithiocarbamate, but none at such a great dilution or for so long a time as this one-hundredth of one per cent of pinosylvin.

Pinosylvin is not now commercially available, but stilbene (or *trans*-stilbene, Eastman No. 1817) $C_6H_5CH{:}CHC_6H_5$, can be readily obtained. Its odor is not that of pine wood, but to the entomologist is reminiscent of a cotton field of which the leaves are being mass devoured by *Alabama* caterpillars. It is not so instantaneously soluble as is pinosylvin, but can be dissolved in benzol or acetone, making a colorless solution for the impregnation of wood. The treated samples of flamboyán wood, even at 2%, are odorless, and unchanged in appearance. The 0.01% sample was toxic to termites for several weeks following impregnation, and although eaten by termites less than three months later, all these termites died. Fresh termites have not enlarged this feeding-gouge, and indeed all have died rather than eat the wood with 0.01% stilbene.

It should not be supposed that all the extractives of pine are similarly toxic or repellent to termites. Chrysin (5,7-dihydroxyflavone) and pinocembrin (5,7-dihydroxyflavonone), extracted by Dr. Gösta Lindstedt from sugar pine, although at first repellent, in less than three months are eaten: the 1% of chrysin and the 0.1% of pinocembrin. At the same time, the greatest dilutions of taxifolin (3,5,7,3′, 4′-pentahydroxy-flavonone), from Douglas fir, and pinosylvin monomethyl ether (3-hydroxy-5-methoxystilbene) and dihydroxypinosylvin monomethyl ether (3-hydroxy-5-methoxydibenzyl) from Scotch pine, have not been attacked in five months after receipt for test.

The permanence of the protection afforded against termite attack is really of more fundamental importance in judging of the value of a chemical than its initial toxicity. Thus, although stilbene and the pinosylvin extractives from Scotch pine may indeed eventually prove to be all that their early promise indicates, the nearly seven years of proved resistance of 0.2% copper pentachlorphenate, and 2% pentachlorphenol and 2% DDT can not be lightly dismissed when compared with the value of chemicals more recently made available. Tests are being conducted at Río Piedras, and it

is hoped that they will continue to be conducted, until some of the more promising of the newer chemicals have had ample time to prove their value.

As compared with DDT for instance, the butyl DDT at 2% impregnation was attacked in 120 days, and the fluorine DDT in 161 days, but at 0.05%, the methyl DDT (duPont's Methoxychlor or Marlate) is still uneaten a year after treatment: a far better record than any of the other benzol-soluble compounds previously reported.

Dimethyl-tetrachlor-phthalate, from the Whitemarsh Laboratories, at this concentration has been uneaten a year and a half, as is also the sample treated with the plant extractive Ryanodine, from *Ryania speciosa* wood from Venezuela. The Laurel Hill Laboratories have submitted samples of somewhat similar compounds, one of which at 0.05% has not been attacked in a year and a half, while others of the series, with but slightly differing composition, are definitely inferior. Such differences in the reactions of termites (and other insects), are possibly to be expected after observing those shown to the gamma isomer of benzene hexachloride (Wolcott 1950), as compared with the lack of protective power possessed by its other isomers.

The constituents of Commercial Solvents' Dilan, and Naugatuck's Aramite show up equally well in preliminary tests. Somewhat disappointing are the records of the Hyman products, so promising at first, but ceasing to protect at 0.2% of impregnation after two years.

TABLE 1

Days after Submersion Ten Minutes in Solution before Attack by the West Indian Dry-Wood Termite, Cryptotermes brevis *Walker*

Dilutions of	0.01%	0.02%	0.05%	0.1%	0.2%	0.5%	1%	2%
Pinosylvin	toxic for two months, inconclusively eaten in five months							
Stilbene	toxic three weeks, inconclusively eaten in three months							
Taxifolin	uneaten in three months							
Pinosylvin monomethyl ether	uneaten in three months							
Dihydropinosylvin monomethyl ether	uneaten in three months							
Pinocembrin (5,7-dihydroxy-flavonone)	57	72	74	78	102	107	uneaten in 5 months	
Chrysin (5,7-dihydroxyflavone)	55	56	58	61	79	82	90 End	
Tectoquinone (Eastman)	3	6	24	36	46	596	uneaten 6 years	
α-naphthaflavone			55	58	60	uneaten in 5 years		
Ryanodine (Merck)	toxic two months, eaten after 204 days slightly eaten in three years							

TABLE 1—*Continued*

Dilutions of	0.01%	0.02%	0.05%	0.1%	0.2%	0.5%	1%	2%
Copper pentachlorphenate (Monsanto)	27	42	108	111	uneaten seven years			
Benzene hexachloride:								
α and β (Eastman)			4	5	7	10	15	44 End
γ (duPont)	136	141	147	189	249	259	303	End
ordinary DDT (Bureau of Entomology)			25	27	29	35	37	uneaten 7 years
Butyl DDT (Whitemarsh)			49	51	79	114	119	120 End
Fluorine DDT (Whitemarsh)			7	50	58	151	157	161 End
Methyl DDT (duPont's Methoxychlor)			uneaten over a year					
Dimethyl-tetrachlorphthalate (Whitemarsh)			uneaten a year and a half					
1,1-di (2-hydroxy-3,5-dichlorphenyl) 2,2,2-trichlorethane (Laurel Hill)			uneaten two years					
1,1-di (2-hydroxy-2,5-dichlorphenyl) 2,2,2-trichlorethane (Laurel Hill)			36	uneaten one year				
1,1-di (4-hydroxy-3,5-dichlorphenyl) 2,2,2-trichlorethane (Laurel Hill)			147	192	330	454	514	End
Dilan (Commercial Solvents): 2-nitro-1,1-bis (p-chlorophenyl) propane			74	83	240	uneaten one year		
butane			84	335	uneaten one year			
Aramite (Naugatuck)			27	80	241	uneaten one year		
Chlordan			20	22	25	48	373	378 End
Dieldrin (Hyman 497)			237	377	473	uneaten two years		
Aldrin (Hyman 118)			363	415	572	uneaten two years		
Toxaphene (Hercules Powder)			150	296	298	300	484	End
Piperonyl Butoxide			19	23	31	181	229	289 End
Cyclohexanone (U. S. Industrial Chemicals)			17	23	157	219	uneaten three years	

On the basis of results to date, certain recommendations may be made. Of the organic compounds, methyl DDT is clearly superior to ordinary DDT, and being commercially available, should displace it for wood treatment. As the tests were made with solutions in benzol, it is not to be expected that suspensions or other preparations in water will be as deeply penetrating or as effective as solutions. Benzol was also used as a solvent for 1,1-di(2-hydroxy-3,5-dichlorphenyl) 2,2,2-trichlorethane. The solution of dimethyl-tetrachlor-phthalate in acetone could not be diluted with water

without precipitation, and the acetone solution of ryanodine became milky when dilution with water was attempted. Herein lies the great advantage of copper pentachlorphenate, for although it can not be dissolved satisfactorily in commercial denatured alcohol, under tropical conditions its solution in pure methyl alcohol, acetone or butyl cellosolve has been diluted indefinitely with water without any indication of precipitation. It would appear to be as effective against powder-post beetles as against termites, for after spraying with a 0.2% solution of copper pentachlorphenate, no more powder fell from a basket from which beetles had already begun to emerge.

Of all the inorganic compounds, the water soluble or the water-miscible solution of copper ammonium fluoride (Wolcott 1949) is unquestionably superior in effectiveness. Recent preparations in both liquid and powder form from the Whitemarsh Laboratories do not require the addition of ammonium hydroxide to the water of dilution to prevent the formation of the blue-grey cloudy precipitate. A one pint bottle of the concentrate dilutes to make one hundred gallons of solution which should protect indefinitely against dry-wood termite attack any wood submerged in it.

With such a variety of effective chemicals for protecting wood, it is now possible to pick and choose, selecting on the basis of cost, or of suitability for the specific use to be made of the impregnated wood. Visual proof of the application of copper ammonium fluoride is afforded by the subsequent blue-grey appearance of light-colored woods, and naturally this chemical should not be used where such a change in the appearance of the wood is undesirable. The dinitro compounds stain the wood a bright yellow, a stain which is also imparted to other objects in contact with the treated wood, but also an unmistakable proof of impregnation. Most of the other effective chemicals give no such visual indication, which is another way of saying that they do not stain the wood, but the proof of their presence lies in the years of service to be expected from such treated woods, free from the danger of early destruction by dry-wood termites.

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EFFECT OF MORA (MORUS ALBA) L. ON ALLOXAN DIABETIC RATS[1]

CONRADO F. ASENJO AND DELIA FERNÁNDEZ[2]

The leaf of *Morus alba* L., commonly known as white mulberry, is widely used in Puerto Rico in the folk treatment of diabetes mellitus. The drug is administered orally in the form of an aqueous infusion several times per day.

A perusal of the literature on mulberry reveals that another species of this genus, *Morus nigra* L., is used in the Balkan countries as a folk remedy for diabetes, in the same manner that *M. alba* is used in Puerto Rico (1).

Bart (2) studied the hypoglycemic effect of aqueous infusions of *M. nigra* leaves on the blood of normal human subjects and found that maximum hypoglycemic action occurred 2 hours after the oral administration of the infusion.

No reference has been found in the literature regarding the use of *M. alba* in the treatment of diabetes mellitus, except the recent clinical investigation by Paniagua and Hernández (3) from our School, to which we shall refer later.

It is the purpose of the present communication to describe experiments done to determine if an aqueous infusion of *M. alba* leaves, prepared as it is commonly used in the folk medicine of Puerto Rico, has hypoglycemic activity when administered, *per os*, to alloxan diabetic rats.

Induction of diabetes in rats by the intravenous injection of alloxan. Young albino rats from our colony (Wistar strain) were used. They were maintained on our stock colony ration before and after injections.[3] These animals ranged between 40–80 grams in weight at the time of injection. A 3 per cent solution of alloxan monohydrate was prepared in sterile distilled water immediately before injection.

In general the technic of Mann and Stare was used (4). The animal was placed in a mailing carton with the tail protruding through a hole in the center of a cork stopper used to close the carton. After cleaning the tail and giving a brisk rub with a dry cloth, or with a small amount of xylol when the veins were difficult to see, the needle was introduced level up, directly over the vein and on the distal one third of the tail. When blood appeared at the syringe nipple, the calculated volume of alloxan solution

[1] Cooperative project between the Agricultural Experiment Station of the University of Puerto Rico and the School of Tropical Medicine.

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[3] Rockland rat diet.

was injected. The dose of alloxan used was 60 mg. per kilo of body weight. This dose was recommended by Mann and Stare (4) as the most practical one to use, as it gives diabetic response in about 90% of the injected animals without causing injury to other organs than the pancreas.

In a period of 2 to 3 days the responding animals developed characteristic diabetic signs, such as hyperglycemia, glycosuria and polyuria.

Preparation of the infusion. Fresh leaves of *Morus alba* L. were obtained from a tree at the Agricultural Experiment Station at Río Piedras, P. R. The infusion was prepared by boiling approximately 100 grams of fresh leaves in 1500 ml. of distilled water for a period of about half hour. This infusion contained 0.5 grams of solids per 100 ml. and no detectable amount of reducing sugars.

Assay of the mora infusion. The mora infusion was administered in place of water for a period of 6 consecutive days. On the 5th day the food was removed and the animals fasted for a period of 24 hours. At the end of this

TABLE 1

Blood sugar of alloxan diabetic rats under different treatments

	Stock colony ration	After 24 hrs. fast receiving H_2O	After 24 hrs. fast receiving mora infusion	After 24 hrs. fast and 2 units of insulin on the 22nd hour
Number of observations	18	10	11	8
Blood sugar mg. per mm^3	394.5	132.4	122.5	73.2
Standard deviation	±50.5	±27.7	±28.7	±23.6

asting period, a blood sample was taken from the tail and glucose was determined using the micro method of Folin and Malmros (table 1). During the last four days of the trial, records were kept of the daily fluid consumption as well as of the total excretion of urinary glucose (table 3).

Hypoglycemic action of insulin. After finishing the trials, 2 units of insulin[4] per kilogram of body weight were injected intraperitoneally, as a check, to eight of the diabetic rats. The rats thus injected had previously fasted for a period of 22 hours. Two hours later blood for glucose determination was taken from the tail of the animals.

RESULTS

Although the 24 hours fasting blood sugar of the mora treated rats was lower than that of the untreated ones, 122.5 and 132.4 mg. per cent, respectively (table 1), the difference between these two values was not statistically significant (table 2).

On the other hand, the injection of 2 units of insulin to the diabetic

[4] Insulin Mulford.

animals previously fasted for a period of 22 hours, induced a significant decrease in the blood sugar 2 hours later; this value was 73.2 mg. per cent.

TABLE 2

Significance of differences observed between the blood sugars of the rats in table 1 that received different treatments

	Difference	$\sigma_{diff.}$*	Degrees of freedom	t†	Remarks
Difference between fasted diabetics receiving H_2O and fasted diabetics receiving mora infusion.	9.9	18.3	19	0.54	Difference not significant
Difference between fasted diabetics receiving H_2O and fasted diabetics receiving H_2O and 2 units of insulin per kilogram of body weight.	59.2	18.3	16	3.24	Difference highly significant
Difference between fasted diabetics receiving mora infusion and fasted diabetics receiving H_2O and 2 units of insulin per kilogram of body weight.	49.3	18.3	17	2.69	Difference significant

* $\sigma_{diff.} = \sqrt{\left(\frac{\Sigma(x_1 - m_1)^2 + \Sigma(x_2 - m_2)^2}{(n_1 - 1) \qquad (n_2 - 1)}\right)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$

† $t = \frac{m_1 - m_2}{\sigma_{diff.}}$

TABLE 3

Daily consumption of fluids and excretion of urinary glucose by alloxan diabetic rats

	On H_2O	On Mora Infusion
(On the stock colony ration)		
Fluid intake ml./24 hrs.	36.7	36.7
Urinary glucose gm./24 hrs.	0.77	0.88
(After 24 hrs. fast)		
Fluid intake ml./24 hrs.	5.9	8.9
Urinary glucose gm./24 hrs.	0.12	0.12

Another index that pointed to the ineffectiveness of the mora infusion was the 24 hour fasting urine glucose excretion. This remained the same in the treated as in the untreated animals: 0.12 grams per day (table 3).

CONCLUSIONS

These results indicate that, under the conditions of our experiment, an aqueous infusion of mora leaf, prepared as previously described, does not

have hypoglycemic effect on alloxan diabetic rats nor does it reduce the excretion of glucose in the urine.

Our findings agree with those of Paniagua and Hernández (3). These investigators submitted to clinical test the same mora leaf infusion prepared by us as already described. Their test consisted of the oral administration of 1000–1500 ml. of the infusion each 24 hour period, for no less than 2 days, to diabetic patients standardized on diets supplying from 150–250 grams of carbohydrate per day. They did not observe improvement in a single one of the 5 patients thus treated. In all of them the blood sugar increased when mora infusion *per os* replaced regular insulin treatment.

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